ARTICLE

OBC
www.rsc.org/obc

# Modulating the activity of oligonucleotides by carbohydrate conjugation: solid phase synthesis of sucrose-oligonucleotide hybrids

**Matteo Adinolfi, Lorenzo De Napoli, Giovanni Di Fabio, Alfonso Iadonisi and Daniela Montesarchio ***

*Dipartimento di Chimica Organica e Biochimica, Università degli Studi di Napoli "Federico II", Complesso Universitario di Monte S. Angelo, via Cynthia, 4, I-80126 Napoli, Italy*

***Received 6th February 2004, Accepted 27th April 2004***
***First published as an Advance Article on the web 7th June 2004***

In order to expand the repertoire of available oligosaccharide-oligonucleotide hybrids, the on-line solid phase synthesis of oligonucleotides conjugated at the 3′- and/or 5′-end with a preformed disaccharide unit has been performed. The key compound in the synthetic scheme described here is an appropriate phosphoramidite derivative of fully protected sucrose, used in association with a solid support functionalized with DMT-protected sucrose. The sucrose units at both ends of selected oligonucleotide sequences were shown to increase their chemical and enzymatic stability, while not interfering with duplex formation and with the ability of G-rich sequences to adopt a quadruplex structure.

## Introduction

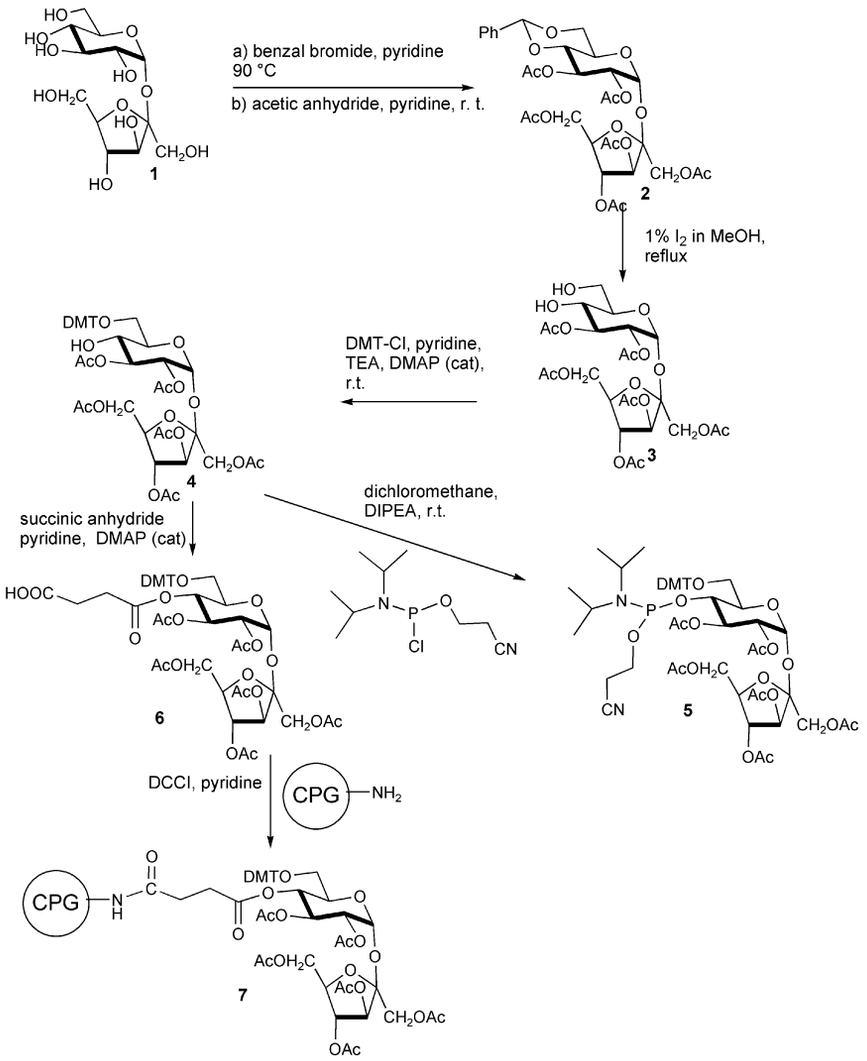
Since more than a decade ago, oligonucleotides (ODNs) of specific sequence have been recognized as highly efficient and selective gene expression inhibitors in antisense[1] or antigene[2] *in vitro* experiments. In addition, interesting, non-antisense biological activities recently emerged also in specific short nucleic acid sequences, called aptamers, acting as efficient protein-binding agents.[3] Unfortunately the therapeutic potential of unmodified ODNs is dramatically limited by their poor cellular uptake and lack of stability against enzymatic degradation. Under *in vivo* conditions they are rapidly hydrolyzed by nucleases and then cleared from the body through renal excretion. Therefore intense research activity in this field has produced a vast number of ODNs bearing chemical modifications in the backbone, mainly at the sugar moiety or at the phosphodiester linkages, in order to assure a prolonged *in vivo* half-life, still preserving if not improving the hybridization capabilities of the oligomers *vs.* the target complementary sequences.[4] As an alternative to the complete replacement of the natural ribose-phosphate skeleton with different, more stable moieties, conjugation at the 3′- and/or 5′-end of ODNs with appropriate molecules imparting specific physico-chemical properties has often resulted in a successful approach, showing several advantages.[5,6] Among the various carriers attached to ODNs to putatively improve their pharmacological profile, carbohydrates may give rise to particularly useful conjugates for targeting cells or tissues with DNA fragments. In fact, not only nuclease resistance and internalisation can be increased, but also aggregation processes can be greatly reduced. As a matter of fact, it is well documented that cell surface sugar binding receptors (lectins) specifically bind and internalise glyco-conjugates bearing appropriate sugar residues.[7,8] Numerous studies have been carried out in the field of glycobiology by conjugating peptides to carbohydrate-based ligands, thus exploiting carbohydrate–protein interactions in order to enhance cellular uptake and drug delivery processes.[9–11] On the contrary, only a few examples of glyco-conjugates of DNA have been reported in the literature. In these syntheses, carbohydrates have been profitably introduced either to increase the bioavailability of ODN.[12–18] or as scaffolds for producing artificial spatially-controlled glycoclusters.[19–22] Indeed, despite the many efforts currently addressed to the solid phase synthesis of oligosaccharides, the regio- and stereocontrolled sequential addition of sugar residues linked by glycosidic junctions is still a very challenging task.[23,24] As a consequence, the preparation of oligonucleotides conjugated to oligosaccharide chains is very far from being optimised.

In our ongoing studies on the on-line solid phase synthesis of modified oligonucleotides, we recently reported a straightforward protocol for the insertion of one glucose moiety at the 5′-OH end of oligonucleotides by direct solid phase glycosidation, using a trichloroacetimidate donor sugar in the presence of TMSOTf.[25,26] The applicability of this extremely practical and simple method is rather limited to oligopyrimidine sequences, since the acidic conditions required for trichloroacetimidate activation, though very mild, still induced some depurination. In addition, the successive introduction of other sugar residues on the polymer-supported chain, with the aim of serially attaching glucose moieties to ODN chains, was not explored, appearing intrinsically cumbersome and low-yielding. Therefore we developed an alternative approach, based on the usage of an appropriate glucose phosphoramidite derivative, which can be incorporated at the last stage of the automated oligonucleotide synthesis.[27] Within this strategy, it is possible to exploit efficient and high fidelity reactions, based on the phosphoramidite chemistry, to generate hydrolytically, enzymatically and thermally stable phosphodiester linkages tethering the saccharide residues to one or both ends of the oligonucleotide. The main advantages of a phosphoramidite-based chemical strategy as an entry to glyco-conjugates of ODNs can be summarized as follows: 1) the same chemistry is exploited to elongate both the carbohydrate and the oligonucleotide domains, so that a unique, on-line solid phase synthetic protocol can be easily and profitably adopted; 2) several saccharidic residues can be sequentially coupled with high efficiency, since the reactivity of the corresponding phosphoramidites proved to be similar to that exhibited by a standard nucleoside 3′-phosphoramidite, with typical coupling yields superior to 98%; 3) the entire oligomer, including the saccharidic surrogate domain, can be assembled by an automated process; 4) the presence of the sugar-phosphate tail was shown to protect the ODNs from nuclease degradation, while not altering the normal hybridization properties of the selected antisense ODN sequence towards complementary nucleic acid fragments.

The first example of ODN chains conjugated with sugar monomers through a phosphodiester junction has been reported in the literature by Akhtar and coworkers, who



DOI: 10.1039/b401819b



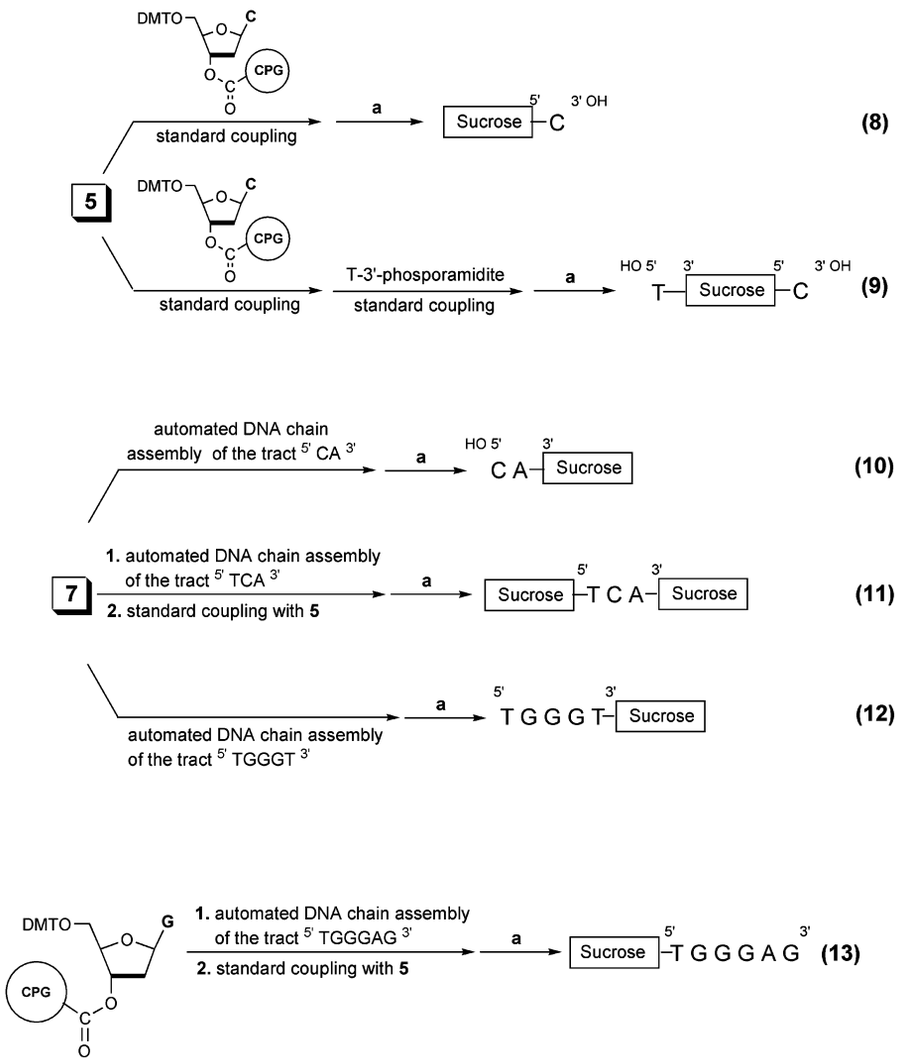

**Scheme 1** Synthetic scheme for the preparation of building block **5** and support **7**.

described the synthesis and use of a mannoside phosphoramidite derivative.[12] Wong *et al*. synthesized ODNs functionalized with glucose- and lactose-phosphate units, baptising this new class of DNA-carbohydrate hybrids 'nucleo-glycoconjugates'.[14] More recently, starting from a phosphoramidite derivative of *N*-acetylglucosamine, Wang and Sheppard successfully prepared a 5′-glucosylated ODN, which was then enzymatically converted into the corresponding Lewis X conjugate.[18]

In an effort to expand the repertoire of easily accessible carbohydrate-oligonucleotide conjugates by exploiting highly efficient phosphoramidite chemistry, we here report the synthesis of ODNs tethering a preformed disaccharide unit either at the 5′- or 3′-end, or incorporated within the chain. As the model disaccharide, we chose sucrose, many derivatives of which were recently found to show multidrug resistance modulator activity.[28] To this purpose, sucrose-functionalized CPG support **7** and protected sucrose phosphoramidite building block **5** have been synthesized in few steps, and used in a typical elongation cycle for the solid phase assembly of oligonucleotides by standard phosphoramidite procedure.[29] To test the feasibility of the proposed synthetic strategy for the preparation of new ODN conjugates, several sucrose-ODN hybrids have been synthesized and fully characterized by $^{1}H$, $^{31}P$ NMR and ESI-MS techniques.

## Results and discussion

The key compound in the synthesis of oligosaccharide-oligonucleotide conjugates described here is saccharide building block **5** (Scheme 1), which was prepared in five steps and 12% overall yield, starting from unprotected sucrose **1**. The synthetic procedure adopted here is essentially similar to the one already described for the synthesis of the corresponding protected glucose 4-phosphoramidite.[27] Sucrose **1** was first converted, in a one-pot reaction, into peracetylated compound **2** in 34% yield by reaction with benzal bromide in pyridine, affording the corresponding 4,6-benzylidene derivative, and subsequent treatment with $Ac_2O$/Py. The successive addition of $I_2$ in $CH_3OH$ allowed the selective removal of the benzylidene protecting group, giving diol **3** in 54% yield. Reaction with DMTCl and cat. DMAP in Py/$Et_3N$ gave desired **4** in 75% yield. This was next phosphitylated by addition of 2-cyanoethyl-*N*,*N*-diisopropylamino-chlorophosphoramidite and DIPEA in anhydrous $CH_2Cl_2$, leading to **5** in 87% yield after silica gel chromatography. To obtain support **7**, intermediate **4** was first succinylated by reaction with succinic anhydride, giving **6** (65% yield). This was then reacted with CPG-$NH_2$ solid support in the presence of DCCI, leading to an average functionalization of 0.07 meq/g, as evaluated by DMT test. All the synthesized compounds were identified on the basis of their $^{1}H$, $^{13}C$ NMR and mass spectral data and found to be pure within the detection limit of the NMR techniques. In the case of phosphoramidite **5**, $^{31}P$ NMR data confirmed its structure as a mixture of diastereomers at phosphorus (see Fig. 1), showing the compound to be pure from the *H*-phosphonate derivative. The homogeneity of sucrose derivatives **5** and **6**, after column chromatography, was also checked by HPLC, which confirmed the compounds to be more than 98% pure.







**Scheme 2** **a**: aq. $NH_4OH$ (conc.), 12 h, 55 °C.

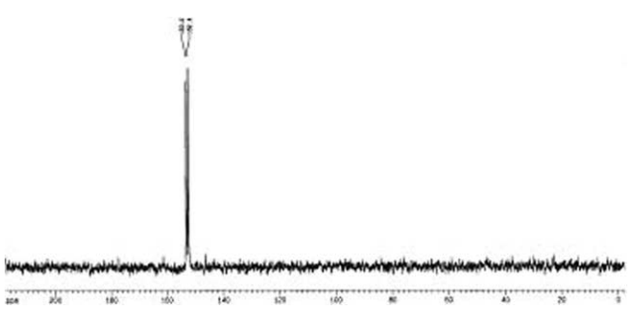


**Fig. 1** $^{31}P$ NMR spectrum (161.98 MHz, $CDCl_3$) of compound **5**.

Building block **5** contains all the typical reactive functional groups necessary in nucleotide monomers for standard ODN assembly *via* the phosphoramidite strategy. So it could be exploited, in connection with the usage of functionalized support **7**, for the synthesis of several hybrids, as models for the preparation of new ODN glyco-conjugates. In all the syntheses, the reactivity of phosphoramidite **5** was similar to that typically found in the coupling with a standard nucleoside 3′-phosphoramidite, *i.e.* in the range 96–98%, as evaluated by spectroscopic DMT test. Hybrids **8**, **9**, **10** and **11**, after HPLC purification, have been fully characterized by $^1H$, $^{31}P$ NMR and ESI MS techniques.

As described in Scheme 2, phosphoramidite **5** was first exploited in a standard coupling with a CPG-bound 2′-deoxycytidine affording, after deprotection and detachment, *sucrose*-p-$^{5'}$C (**8**) as a model compound to mimic the solid phase insertion of one sucrose phosphoramidite residue at the 5′-OH of an oligonucleotide. Then **5** was inserted in the middle of a short sequence, leading to $^{5'}$T-p-*sucrose*-p-C$^{3'}$ (**9**). Starting from support **7**, as a test for 3′-conjugation of ODN fragments, two standard couplings with nucleoside 3′-phosphoramidites were carried out, obtaining $^{5'}$C-p-A$^{3'}$-p-*sucrose* (**10**), which confirmed the high efficiency of coupling with the 6-OH function of the glucose residue in the polymer-supported disaccharide. The combined use of **5** and support **7** allowed the preparation of 3′,5′-bis-conjugated trinucleotide *sucrose*-p-$^{5'}$T-p-C-p-A$^{3'}$-p-*sucrose* **11**. Also in the case of 3′,5′-conjugation, the usual yields were observed, as calculated by colorimetric DMT test during the chain assembly.

All the syntheses of the ODNs were carried out on a 5 μmol scale by automated solid phase protocols, by employing slightly modified phosphoramidite procedures.[29] The final detachment from the solid support and deprotection of the conjugated oligomers were achieved by conc. aq. ammonia treatment at 55 °C for 12 h. The synthesized ODNs were then purified by HPLC on an analytical anionic exchange column. As an example, the HPLC profile of crude **11** is shown in Fig. 2 (panel A). The collected peaks were then desalted by gel filtration chromatography on a Sephadex G25 column eluted with $H_2O$/EtOH 4 : 1 (v/v). The purity of the compounds was then checked by RP-HPLC and their identity ascertained by $^1H$, $^{31}P$ NMR and ESI-MS analysis.

The chemical stability of the sucrose conjugates was analysed by incubating 1.0 OD of purified hybrid **11** at 37 °C in 1.0 mL of a phosphate buffer (20 mM $KH_2PO_4$ aq. solution, pH 7.0, containing 20% (v/v) $CH_3CN$ at pH = 7.0); no degradation was observed even after 10 d in the HPLC profile on an analytical

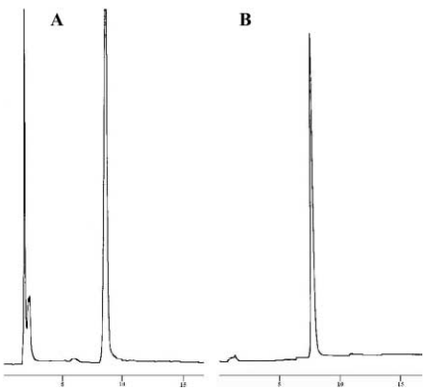



**Fig. 2** **A**: HPLC profile of crude **11** (Nucleogel SAX column – Macherey-Nagel, 1000-8/46, linear gradient from 0 to 100% B in 30 min, flow rate 0.8 mL $min^{-1}$, of buffer A: 20 mM $KH_2PO_4$ aq. solution, pH 7.0, containing 20% (v/v) $CH_3CN$, in $H_2O$); **B**: HPLC profile of purified **11** after 10 days of incubation at 37 °C in a phosphate buffer (20 mM $KH_2PO_4$ aq. solution, pH 7.0).

anionic exchange column (Fig. 2, panel B). Compound **11** was also completely stable in biological media: when incubated at 37 °C in bovine fœtal serum, no hydrolysis or degradation products were found in the mixture, analysed by HPLC after 10 d. For comparison, unconjugated trimer $^{5'}$TCA$^{3'}$, treated under the same conditions, was completely degraded after 4 h of incubation.

We next studied the influence of sucrose units at the ends of oligonucleotide chains on their recognition properties, due to specific H-bonding abilities. As a first experiment, G-rich sequences were chosen as valuable substrates for studying the effect of this conjugation, in view of their well known propensity to adopt stable G-quadruplex structures.[30,31] In fact they are highly represented in telomers and are responsible for telomer stabilization and therefore deeply involved in fundamental cellular processes as senescence. Particularly, $^{5'}$TGGGT$^{3'}$,[32] and $^{5'}$TGGGAG$^{3'}$,[33] have both been demonstrated to give stable parallel four stranded quadruplex complexes. Interestingly, as a consequence of their characteristic structure, many 5′-derivatives of $^{5'}$TGGGAG$^{3'}$ have displayed a significant biological activity as non antisense anti-HIV agents.[33] These model sequences have therefore been conjugated with one sucrose unit *via* a phosphodiester linkage, either to the 3′- or 5′-end, giving oligomers **12** and **13**, respectively. In order to verify that the ability of 5-mer $^{5'}$TGGGT$^{3'}$ and 6-mer $^{5'}$TGGGAG$^{3'}$ to form quadruplex structures was not inhibited when a sucrose tail was attached at either end of the oligonucleotide sequence, a CD analysis was undertaken on the conjugated *sucrose*-p-$^{5'}$TGGGAG$^{3'}$ and $^{5'}$TGGGT$^{3'}$-p-*sucrose* in comparison with the corresponding unmodified oligomers. It has been found that the conformational behaviour of glyco-conjugates **12** and **13** in the buffer 10 mM $KH_2PO_4$, 1.00 M KCl and 0.1 mM EDTA, where typically G-rich sequences form quadruplexes, is qualitatively very similar to one of the parent oligomers. Particularly, in all the spectra a large positive band at 260 nm and a negative band at 240 nm were apparent (see Fig. 3), indicative of a parallel four stranded structure, stabilized by a set of specific H-bond arrays.[30–33] Interestingly, at 20 °C the CD profile of **13** showed an increased ellipticity in the bands at 260 nm and at 240 nm in comparison with the parent 6-mer, thus showing that, upon insertion of one sucrose-phosphate tether at the 5′-end of the oligomer, a stabilization of the quadruplex structure occurred, in analogy with other 5′-derivatives of the same sequence described in the literature.[33] On the contrary, conjugation at the 3′-end caused a drop in the positive and negative Cotton effects, at 260 nm and at 240 nm respectively, indicating the presence of a less stable quadruplex structure in **12** with respect to the unmodified oligomer. Further studies, involving NMR and DSC analyses, are

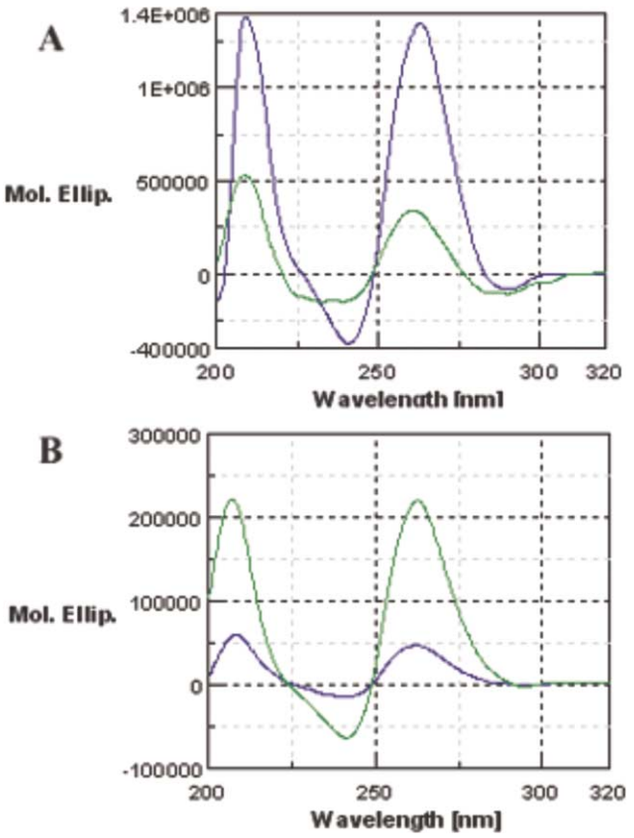


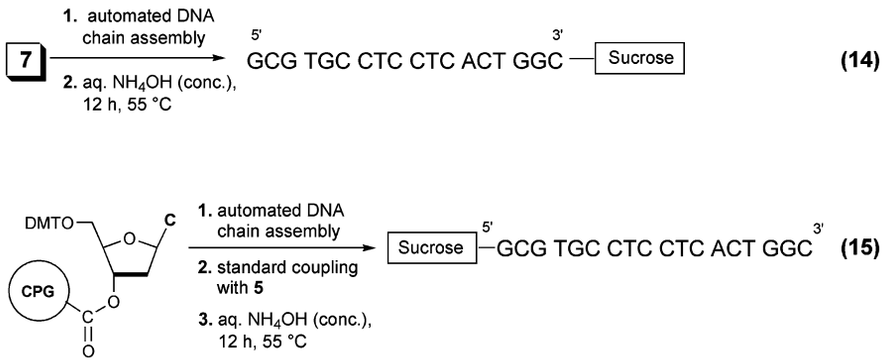

**Fig. 3** **A**: CD spectra of glycoconjugate **13** (blue line) and of 6-mer $^{5'}$TGGGAG$^{3'}$ (green line) at $3.3 \times 10^{-5}$ M at 20 °C; **B**: CD spectra of glycoconjugate **12** (blue line) and of 5-mer $^{5'}$TGGGT$^{3'}$ (green line) at $1.0 \times 10^{-4}$ M at 20 °C.

currently underway to get a deeper insight into the structural details of the synthesized quadruplex structures.

Successively, we investigated the duplex formation ability of sucrose-conjugates of ODNs. Two 18-mers of sequence d($^{5'}$GCGTGCCTCCTCACTGGC$^{3'}$),[34] having one sucrose-phosphate residue either at the 3′ or at the 5′-end (**14** and **15**, Scheme 3), were therefore synthesized following a standard automated phosphoramidite procedure. After ammonia deprotection and HPLC purification, the conjugated ODNs were characterised by MALDI-TOF mass spectrometry, giving in both cases a single peak in accordance with the calculated molecular weight. In order to evaluate the influence of the sugar-phosphate tethers on the hybridization properties of the synthesized ODNs, thermal denaturation studies were carried out mixing glyco-conjugates **14** or **15** in 1 : 1 ratio with complementary 22-mer d($^{5'}$TTGCCAGTGAGGAGGCACG-CAT$^{3'}$). The melting curves of both modified duplexes were almost superimposable with that of the natural, unmodified duplex ($T_m$ = 60 °C), thus showing that the conjugation described here does not negatively interfere with the hybridisation properties of ODNs.

## Conclusions

In this paper we have synthesized, exploiting an on-line solid phase procedure, new oligonucleotide hybrids, conjugated with preformed sucrose units linked to the 3′ and/or 5′ OH groups of the ODN chain through stable phosphodiester junctions. The synthetic protocol we have developed is based on the preparation of a stable phosphoramidite derivative of suitably protected sucrose (**5**) and on the usage of a polymeric support *ad hoc* functionalized with a DMT-protected sucrose residue (**7**). In all cases, **7** and **5** exhibited the typical efficiency in coupling with standard nucleosides 3′-phosphoramidite or with standard functionalized CPG supports (*i.e.* 98% on average),




**Scheme 3**

which allowed the synthesis of several hybrid molecules. The presence of the sucrose tail was shown not to inhibit the ability of G-rich sequences to adopt typical quadruplex structures, as demonstrated by CD studies, neither of mixed sequence ODNs to form stable duplexes with complementary DNA strands, as evaluated by UV thermal denaturation studies. The disaccharide tail significantly protected oligonucleotides from chemical and enzymatic degradation.

## Experimental

### Materials and methods

NMR spectra were recorded on Bruker WM-400, Varian Gemini 300 and Varian Inova 500 spectrometers. All chemical shifts are expressed in ppm with respect to the residual solvent signal. The solid support functionalizations were carried out in a short glass column (5 cm length, 1 cm i.d.) equipped with a sintered glass filter, a stopcock and a cap. The oligonucleotides were assembled on a Millipore Cyclone Plus DNA synthesizer, using commercially available 3′-O-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite 2′-deoxyribonucleosides as building blocks.

The following abbreviations were used throughout the text: Controlled Pore Glass (CPG), dichloroacetic acid (DCA), dichloromethane (DCM), *N*,*N*-dicyclohexylcarbodiimide (DCCI), diisopropylethylamine (DIPEA), *N*,*N*-dimethylaminopyridine (DMAP), 4,4′-dimethoxytrityl (DMT), fructose residue of sucrose (Fru), glucose residue of sucrose (Glc), pyridine (Py), triethylamine (TEA), triethylammonium bicarbonate (TEAB), trimethylsilyltriflate (TMSOTf).

HPLC analyses and purifications were performed on a Beckman System Gold instrument equipped with a UV detector module 166 and a Shimadzu Chromatopac C-R6A integrator. By HPLC analysis on a Partisphere Whatman RP18 analytical column (125 × 4.0 mm, 5 μm), eluted with a linear gradient from 0 to 100% in 60 min of $CH_3CN$ in TEAB buffer (0.1 M, pH 7.0), flow = 0.8 mL $min^{-1}$, the isolated oligomers **8**–**15** were determined to be more than 98% pure. For the ESI MS analyses a Waters Micromass ZQ instrument – equipped with an Electrospray source – was used in the positive and/or negative mode. For compounds **9**, **10**, **11**, **12** and **13** the reported mass is the calculated value on the basis of the combination of the found multiple charged ions. MALDI TOF mass spectrometric analyses were performed on a PerSeptive Biosystems Voyager-De Pro MALDI mass spectrometer in the Linear mode using a picolinic/3-hydroxypicolinic acid mixture as the matrix. Optical rotations were measured with a Perkin Elmer 141 polarimeter at 20 °C and are quoted in units of $10^{-1}$ deg $cm^2$ $g^{-1}$. UV measurements and thermal denaturation experiments (detection at $\lambda$ = 260 nm) were carried out on a JASCO V-530 UV spectrophotometer equipped with a JASCO ETC-505T temperature controller unit. CD spectra were obtained on a JASCO 715 circular dichroism spectrophotometer at 20 °C in a 0.1 cm pathlength cuvette. Temperature was kept constant with a thermoelectrically controlled cell holder (JASCO PTC-348).

### Synthesis of phosphoramidite 5

Sucrose (1.50 g, 4.38 mmol) was suspended in pyridine (15 mL) and the mixture was heated to 90 °C. Benzal bromide was added in two aliquots (700 μL each, 4.2 mmol) within two hours. After two hours from the last addition, the mixture was cooled to room temperature, and acetic anhydride (5 mL) was added. After completion of the reaction (TLC analysis), methanol was added at 0 °C to destroy excess acetic anhydride. The mixture was then extracted with DCM and the organic phase was washed with water and taken to dryness. Silica gel chromatography of the residue (eluent petroleum ether/ethyl acetate from 75 : 25 to 70 : 30) furnished compound **2** (1.013 g, yield 34%) as a foam.

**2** – $R_f$ (ethyl acetate/petroleum ether 1 : 1) 0.50; $[\alpha]^{20}_D$ = +45.7 (*c* 0.2 in $CH_2Cl_2$); $\lambda_{max}$ ($CH_2Cl_2$)/nm 258 ($\varepsilon$/$dm^3$ $mol^{-1}$ $cm^{-1}$ 371); $^1$H NMR data of **2** were identical to those previously reported.[35]

$^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 170.6, 170.3, 170.2, 170.2, 169.8, and 169.8 (6 × $COCH_3$); 136.9 (aromatic quaternary C); 129.0, 128.2 and 126.1 (aromatic CH); 104.0 (C-2 Fru); 101.6 (benzylic CH benzylidene); 90.4 (C-1 Glc); 79.1, 78.8, 75.5, 74.7, 71.0, 68.7, 68.4 (C-3 Fru, C-4 Fru, C-5 Fru, C-2 Glc, C-3 Glc, C-4 Glc and C-5 Glc); 63.5, 63.3 and 63.1 (C-1 and C-6 Fru, C-6 Glc); 20.6 ($COCH_3$).

ESI-MS (positive ions): calculated mass for $C_{31}H_{38}O_{17}$: 682.211; found: *m*/*z* 705.35 $(M + Na)^+$; 721.29 $(M + K)^+$.

Compound **2** (1.01 g, 1.48 mmol) was dissolved in a solution of iodine in methanol (1%, 27.5 mL). The resulting mixture was refluxed for 6 h and then sodium thiosulfate was added at room temperature until the iodine was consumed. The mixture was extracted several times with ethyl acetate and the organic phase concentrated under reduced pressure. Silica gel chromatography of the residue (eluent ethyl acetate/hexanes 85 : 15) afforded pure diol **3** as a foam (474 mg, yield 54%).

**3** – $R_f$ (ethyl acetate/petroleum ether 9 : 1) 0.50; $[\alpha]^{20}_D$ = +56.4 (*c* 0.4 in $CH_2Cl_2$); $\lambda_{max}$ ($CH_2Cl_2$)/nm 252, shoulder ($\varepsilon$/$dm^3$ $mol^{-1}$ $cm^{-1}$ 109); $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 5.62 (1H, d, $J_{1,2}$ = 3.6 Hz, H-1 Glc); 5.44 (1H, d, $J_{3,4}$ = 5.6 Hz, H-3 Fru); 5.37 (1H, t, $J_{3,4} = J_{4,5}$ = 5.6 Hz, H-4 Fru); 5.32 (1H, t, $J_{2,3} = J_{3,4}$ = 9.2 Hz, H-3 Glc); 4.76 (1H, dd, H-2 Glu); 4.52–4.18 (5H, overlapped signals, $H_2$-1 Fru, H-5 Fru, and $H_2$-6 Fru); 3.98 (1H, m, H-5 Glc); 3.90 (1H, dd, $J_{5,6a}$ = 3.0 Hz, $J_{6a,6b}$ = 12.6 Hz, H-6a Glc); 3.79 (1H, dd, $J_{5,6b}$ = 5.0 Hz, $J_{6a,6b}$ = 12.6 Hz, H-6b Glc); 3.63 (1H, t, $J_{3,4} = J_{4,5}$ = 9.2 Hz, H-4 Glc); 2.16, 2.10, 2.10, 2.09, 2.09 and 2.09 (3H each, s's, 6 × –$COCH_3$).

$^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 171.2, 171.0, 170.3, 170.2, 170.1 and 169.9 (6 × $COCH_3$); 103.8 (C-2 Fru); 90.0 (C-1 Glc); 78.9, 75.6, 74.9, 72.8, 72.2, 70.4 and 69.2 (C-3 Fru, C-4 Fru, C-5 Fru, C-2 Glc, C-3 Glc, C-4 Glc and C-5 Glc); 63.9, 62.8 and 61.8 (C-1 and C-6 Fru, C-6 Glc); 20.6 ($COCH_3$).

ESI-MS (positive ions): calculated mass for $C_{24}H_{34}O_{17}$: 594.180; found: *m/z* 617.30 $(M + Na)^+$; 633.24 $(M + K)^+$.

Compound **3** (450 mg, 0.76 mmol) and DMAP (4.5 mg, 0.038 mmol) were dissolved in 6 mL of anhydrous pyridine. To the stirred solution, DMTCl (282 mg, 0.83 mmol) and TEA (120 μL, 0.85 mmol) were sequentially added and the mixture was left overnight under stirring and then concentrated under reduced pressure. Silica gel chromatography of the residue (eluent petroleum ether/ethyl acetate 1 : 1) afforded compound **4** as a white foam (513 mg, yield 75%).

**4** – $R_f$ (ethyl acetate/petroleum ether 3 : 2) 0.70; $[\alpha]^{20}_D = +38.4$ (*c* 0.2 in $CH_2Cl_2$); $\lambda_{max}$ ($CH_2Cl_2$)/nm 274 ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$ 2667); $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 7.40–6.80 (13H, complex signals, aromatic protons); 5.63 (1H, d, $J_{1,2}$ = 3.6 Hz, H-1 Glc); 5.41 (1H, d, $J_{3,4}$ = 5.6 Hz, H-3 Fru); 5.37 (1H, t, $J_{2,3} = J_{3,4}$ = 10.2 Hz, H-3 Glc); 5.29 (1H, t, $J_{3,4} = J_{4,5}$ = 5.6 Hz, H-4 Fru); 4.79 (1H, dd, H-2 Glu); 4.40–4.05 (7H, overlapped signals, H-4 Glc, H-5 Glc, $H_2$-1 Fru, H-5 Fru and $H_2$-6 Fru); 3.77 (6H, s, 2 × $OCH_3$); 3.36 (2H, d, $J_{5,6a} = J_{5,6b}$ = 3.8 Hz, $H_2$-6 Glu); 2.10, 2.10, 2.09, 2.08, 2.08 and 2.01 (3H each, s's, 6 × $COCH_3$).

$^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 170.8, 170.6, 170.4, 170.0, 170.0 and 169.7 (6 × $COCH_3$); 158.4, 144.5, 135.6 (aromatic C DMT group); 129.9, 129.0, 128.0, 127.7, 126.6 and 113.0 (aromatic CH DMT group); 104.2 (C-2 Fru); 90.4 (C-1 Glc); 86.2 (non aromatic quaternary carbon of DMT group); 79.3, 75.6, 75.2, 71.9, 71.3, 70.6 and 70.0 (C-3 Fru, C-4 Fru, C-5 Fru, C-2 Glc, C-3 Glc, C-4 Glc and C-5 Glc); 63.9, 62.7 and 62.4 (C-1 and C-6 Fru, C-6 Glc); 55.0 ($OCH_3$ of DMT group); 20.6 ($COCH_3$).

ESI-MS (positive ions): calculated mass for $C_{45}H_{52}O_{19}$: 896.310; found: *m/z* 919.41 $(M + Na)^+$; 935.40 $(M + K)^+$.

To 255 mg of compound **4** (0.28 mmol), dissolved in 3 mL of anhydrous dichloromethane, was added DIPEA (150 mL, 0.87 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylamino-chlorophosphoramidite (95 μL, 0.42 mmol) under argon. After 30 minutes the solution was diluted with ethyl acetate and the organic phase was washed twice with brine and then concentrated. Silica gel chromatography of the residue (eluent petroleum ether/ethyl acetate from 3 : 2 to 2 : 3) afforded compound **5** as a mixture (*ca.* 1 : 1, as evaluated on the basis of $^1$H and $^{31}$P NMR spectra) of diastereomers (266 mg, yield 87%). Injected on an analytical silica gel HPLC column (Macherey-Nagel Nucleosil 50-7 column, 4.6 × 250 mm, 7 μm) eluted with 35% ethyl acetate in cyclohexane, flow = 0.8 mL min$^{-1}$, detection at $\lambda$ = 260 nm, a sole, sharp peak (retention time 7.45 min) was found.

**5** – Glassy solid. $R_f$ (ethyl acetate/petroleum ether 1 : 1) 0.45; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.49–7.79 (26H, complex signals, aromatic protons); 5.73 (2H, d, $J_{1,2}$ = 4.5 Hz, H-1 Glc); 5.50–5.40 (4H, overlapped signals, H-3 Glc and H-3 Fru); 5.35–5.21 (2H, two apparent t's, *J* = 5.2 and 4.8 Hz, H-4 Fru); 4.88–4.78 (2H, two dd's, H-2 Glc); 4.35–3.93 (16H, overlapped signals, $H_2$-1, H-5 and $H_2$-6 Fru, H-4 and $H_2$-6 Glc); 3.77 (12H, s, $OCH_3$ of DMT group); 3.77–3.51 [6H, overlapped signals, H-5 Glc and two $CH(CH_3)_2$]; 3.38 and 3.22 (2H each, t's, two –$OCH_2CH_2CN$); 2.53 and 2.25 (2H each, t's, two –$OCH_2CH_2CN$); 2.11, 2.10, 2.09, 2.07, 2.00 and 1.98 (36H, s's, six $COCH_3$); 1.04, 0.97 and 0.87 (24H, three d's, *J* = 8.5 Hz, four $CH(CH_3)_2$].

$^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 170.4, 170.3, 170.0, 169.9 and 169.6 ($COCH_3$); 158.2, 144.9, 136.0, 135.9, 135.7, 130.2, 130.1, 128.2, 127.4, 126.5 and 112.7 (aromatic carbons DMT group); 117.4 (CN); 104.3 and 104.2 (C-2 Fru); 90.1 (C-1 Glc); 85.7 (quaternary C of DMT group); 79.4 (C-5 Fru); 75.8, 75.7 and 75.6 (C-3 Fru, C-4 Fru and C-3 Glc); 71.8, 71.6, 70.7 and 70.2 (C-2 Glc, C-4 Glc and C-5 Glc); 63.8, 63.6 and 62.5 (C-1 Fru, C-6 Fru and C-6 Glc); 58.6 and 58.1 ($OCH_2CH_2CN$); 55.0 ($OCH_3$ DMT group); 42.9 and 42.6 [$CH(CH_3)_2$]; 24.3 and 24.2 [$CH(CH_3)_2$]; 21.3, 21.0, 20.5, 20.3, 20.0 and 19.9 ($COCH_3$ and $OCH_2CH_2CN$).

$^{31}$P NMR (161.98 MHz, $CDCl_3$) $\delta$ 153.0 and 152.3.

ESI-MS (positive ions): calculated mass for $C_{54}H_{69}N_2O_{20}P$: 1096.418; found *m/z* 1097.56 $(M + H)^+$; 1120.73 $(M + Na)^+$.

### Synthesis of functionalized support 7

Compound **4** (250 mg, 0.28 mmol) and DMAP (68 mg, 0.56 mmol) were dissolved in anhydrous pyridine and succinic anhydride (50 mg, 0.50 mmol) was added to the resulting solution. The mixture was kept under stirring for 60 hours at room temperature and then concentrated. Silica gel chromatography of the residue (eluent DCM/methanol from 95 : 5 to 9 : 1) afforded **6** as a foam (180 mg, yield 65%). Isolated compound **6**, redissolved in ethyl acetate and injected on an analytical silica gel HPLC column (Macherey-Nagel Nucleosil 50-7 column, 4.6 × 250 mm, 7 μm) eluted with ethyl acetate, flow = 0.8 mL min$^{-1}$, detection at $\lambda$ = 260 nm, gave a sole peak (retention time 7.15 min).

**6** – $R_f$ (DCM/methanol 95 : 5) 0.40; $[\alpha]^{20}_D = +48.7$ (*c* 0.1 in $CH_2Cl_2$); $\lambda_{max}$ ($CH_2Cl_2$)/nm 273 ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$ 2449); $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 7.40–6.85 (13H, complex signals, aromatic protons); 5.75 (1H, d, $J_{1,2}$ = 3.6 Hz, H-1 Glc); 5.50–5.26 (4H, overlapped signals, H-3 Fru, H-4 Fru, H-3 Glc and H-4 Glc); 4.92 (1H, dd, $J_{2,3}$ = 9.4 Hz, H-2 Glc); 4.40–4.10 (6H, overlapped signals, H-5 Glc, $H_2$-1 Fru, H-5 Fru and $H_2$-6 Fru); 3.75 (6H, s, 2 × $OCH_3$); 3.34 (1H, br d, $J_{6a,6b}$ = 10.6 Hz, $H_a$-6 Glc); 2.98 (1H, dd, $J_{5,6b}$ = 3.0 Hz, $H_b$-6 Glc); 2.60–2.30 (4H, m, two $CH_2$ succinic group); 2.10, 2.10, 2.10, 2.07, 2.00 and 1.97 (3H each, s's, 6 × $COCH_3$).

$^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 174.6, 170.5, 170.4, 170.3, 170.3, 170.2, 169.7 and 169.7 (6 × $COCH_3$ and ester succinic CO); 158.3, 144.5, 136.7 (aromatic quaternary C DMT group); 130.0, 128.2, 127.6, 126.6 and 113.0 (aromatic CH DMT group); 104.2 (C-2 Fru); 90.2 (C-1 Glc); 85.8 (non aromatic quaternary carbon of DMT); 79.2, 75.6, 75.2, 70.6, 69.8, 69.7 and 68.4 (C-3 Fru, C-4 Fru, C-5 Fru, C-2 Glc, C-3 Glc, C-4 Glc and C-5 Glc); 63.7, 62.7 and 60.9 (C-1 and C-6 Fru, C-6 Glc); 55.0 (-$OCH_3$); 28.9 and 28.8 (succinic $CH_2$); 20.6 ($COCH_3$).

ESI-MS (negative ions): calculated mass for $C_{49}H_{56}O_{22}$: 996.326; found: *m/z* 995.37 $(M - H)^-$.

To a solution of compound **6** (170 mg, 0.17 mmol) in 2 mL of anhydrous pyridine was added DCCI (144 mg, 0.70 mmol). After 5 min the mixture was cannulated to a vessel containing amino-functionalized CPG resin (240 mg). The mixture was left under gentle vascular stirring for three days and then the resin was filtered and repeatedly washed with dichloromethane and methanol. Treatment of 5 mg of dried resin **7** with perchloric acid/ethanol 3 : 2 and colorimetric assay of the released dimethoxytrytyl cation allowed the determination of the functionalization of the resin, which was 0.07 mmol g$^{-1}$.

### Synthesis of hybrids 8, 9, 10 and 11. General procedure

The syntheses of hybrids **8**, **9**, **10** and **11** have been carried out on an automated DNA synthesizer following standard phosphoramidite chemistry,[29] using for the amidite unit (both standard nucleosides 3′- phosphoramidite and phosphoramidite **5**) a 40 mg mL$^{-1}$ solution in anhydrous $CH_3CN$. 50 mg (3.5 μmol) of support **7** or 50 mg of the appropriate DMT-nucleoside supported CPG (*ca.* 5 μmol) were used as the starting solid support for each synthesis. In all the experiments, incorporation of the sucrose-phosphate residue at the 5′-end or within the chain occurred with yields in the range 96–98% (by DMT test). Once the desired ODN sequence was completed, the solid supports were treated with the DCA solution for the final DMT removal and, after exhaustive washings with DCM and $CH_3CN$, left in contact overnight with a conc. aq. ammonia solution at 55 °C. The filtered solutions and two washings with $H_2O$ were concentrated under reduced pressure and then – for compounds **8**–**10** – purified by HPLC on a Nucleosil 100-5 $C_{18}$ column (4.6 × 250 mm, 7 μm) eluted with a linear gradient from 0 to 25% in 30 min of $CH_3CN$ in TEAB buffer (0.1 M, pH 7.0),

flow = 0.8 mL $min^{-1}$. The final products were lyophilized several times to remove the triethylammonium bicarbonate salt and characterized by NMR and MS data. Crude **11** was purified by HPLC on a Nucleogel SAX column (Macherey-Nagel, 1000-8/46), using a linear gradient from 0 to 100% B in 30 min, flow rate 0.8 mL $min^{-1}$, of buffer A: 20 mM $KH_2PO_4$ aq. solution, pH 7.0, containing 20% (v/v) $CH_3CN$, in $H_2O$, and successively desalted by gel filtration on a Sephadex G25 column eluted with $H_2O$/EtOH 4 : 1 (v/v).

Compound **8**: retention time 10.44 min; $^1$H NMR (400 MHz, $D_2O$), $\delta$ 7.86 (1H, d, $J$ = 7.6 Hz, H-6 C); 6.30 (1H, dd, $J$ = 6.4 and 6.6 Hz, H-1′); 6.08 (1H, d, $J$ = 7.6 Hz, H-5 C); 5.44 (1H, d, $J$ = 4.0 Hz, H-1 Glc); 4.47 (1H, m, H-3′); 4.22 (4H, overlapped signals, H-4′, $H_2$-5′ C and H-4 Glc); 4.11 (1H, m, H-3 Fru); 4.08–3.77 (8H, overlapped signals, H-3, H-5, $H_2$-6 Glc and H-4, H-5 and $H_2$-6 Fru); 3.71 (2H, s, $H_2$-1 Fru); 3.62 (1H, m, H-2 Glc); 2.31 (2H, m, $H_2$-2′).

$^{31}$P NMR (161.98 MHz, $D_2O$), $\delta$ 2.02.

ESI-MS: calculated mass for $C_{21}H_{34}N_3O_{17}P$: 631.481; found: $m/z$ 630.17 $(M - H)^-$.

Compound **9**: retention time 15.20 min; $^1$H NMR (500 MHz, $D_2O$), $\delta$ 7.97 (1H, d, $J$ = 7.5 Hz, H-6 C); 7.67 (1H, s, H-6 T); 6.27 (2H, dd, $J$ = 6.2 and 6.2 Hz, overlapped H-1′ T and C); 6.11 (1H, d, $J$ = 7.5 Hz, H-5 C); 5.43 (1H, d, $J$ = 3.5 Hz, H-1 Glc); H-3′ of T residue is buried under the residual HDO solvent signal; 4.57 (1H, m, H-3′ of C); 4.27–4.13 (7H, overlapped signals, 2 H-4′, $H_2$-5′ of C, H-4 and $H_2$-6 Glc); 4.10 (1H, m, H-3 Fru); 4.02–3.78 (8H, overlapped H-3 and H-5 Glc, H-4, H-5 and $H_2$-6 Fru and $H_2$-5′ of T); 3.67 (3H, overlapped signals, H-2 Glc and $H_2$-1 Fru); 2.62–2.23 (4H, m, $H_2$-2′ T and C); 1.90 (3H, s, $CH_3$ T).

$^{31}$P NMR (161.98 MHz, $D_2O$), $\delta$ 2.89 and 2.35.

ESI-MS: calculated mass for $C_{31}H_{47}N_5O_{24}P_2$: 935.675; found: $m/z$ 934.37 $(M - H)^-$.

Compound **10**: retention time 16.27 min; $^1$H NMR (400 MHz, $D_2O$), $\delta$ 8.48 (1H, s, H-2 A); 8.20 (1H, s, H-8 A); 7.53 (1H, d, $J$ = 8.0 Hz, H-6 C); 6.48 (1H, dd, $J$ = 6.0 and 6.0 Hz, H-1′ A); 6.02 (1H, dd, $J$ = 6.2 and 6.2 Hz, H-1′ C); 5.92 (1H, d, $J$ = 8.0 Hz, H-5 C); 5.27 (1H, d, $J$ = 3.5 Hz, H-1 Glc); 5.01 (1H, m, H-3′ A); 4.76 (1H, m, H-3′ C); 4.48 (1H, m, H-4′ A); 4.22–3.95 (7H, overlapped signals, H-4 and $H_2$-6 Glc, H-3 Fru, H-4′ C and $H_2$-5′ A); 3.88–3.50 (11H, overlapped signals, $H_2$-5′ C, H-2, H-3 and H-5 Glc and $H_2$-1, H-4, H-5 and $H_2$-6 Fru); 2.78 (2H, m's, $H_2$-2′ A); 2.27 (2H, m's, $H_2$-2′ C).

$^{31}$P NMR (161.98 MHz, $D_2O$), $\delta$ 2.55 and 2.01;

ESI-MS: calculated mass for $C_{31}H_{46}N_8O_{22}P_2$: 944.689; found: 943.44 $(M - H)^-$.

Compound **11**: retention time 8.89 min; $^1$H NMR (400 MHz, $D_2O$), $\delta$ 8.49 (1H, s, H-2 A); 8.23 (1H, s, H-8 A); 7.65 (1H, s, H-6 T); 7.58 (1H, d, $J$ = 7.6 Hz, H-6 C); 6.48 (1H, dd, $J$ = 6.0 and 6.0 Hz, H-1′ A); 6.30 (1H, dd, $J$ = 6.0 and 5.8 Hz, H-1′ T); 6.10 (1H, dd, $J$ = 6.0 and 6.0 Hz, H-1′ C); 6.02 (1H, d, $J$ = 7.6 Hz, H-5 C); 5.44 (1H, d, $J$ = 4.0 Hz, H-1 Glc at the 5′-end); 5.33 (1H, d, $J$ = 3.6 Hz, H-1 Glc at the 3′-end); 5.09 (1H, m, H-3′ A); H-3′ C residue and H-3′ T residue are submerged by the residual HDO solvent signal; 4.49 and 4.34 (1H each, m, two H-4′); 4.24–3.53 (20H, overlapped signals, one H-4′ and three $H_2$-5′ A,C,T residues, H-2, H-3, H-4, H-5 and $H_2$-6 Glc, $H_2$-1, H-3, H-4, H-5 and $H_2$-6 Fru); 2.83, 2.45 and 2.29 (2H each, m's, $H_2$-2′); 1.93 (3H, s, $CH_3$ T).

$^{31}$P NMR (161.98 MHz, $D_2O$), $\delta$ 2.86, 2.54, 2.20 and 1.89.

ESI-MS: calculated mass for $C_{53}H_{80}N_{10}O_{42}P_4$: 1652.338; found: 1652.94.

### Synthesis of conjugates 12 and 13 and of the parent oligonucleotides

ODN chain assembly was performed on 50 mg (3.5 μmol) of support **7** for **12**, and on 50 mg of DMT-G-supported CPG (Millipore, High Loading, *ca.* 0.1 meq/g) for **13**, on an automated DNA synthesizer following a standard phosphoramidite protocol with final DMT removal.[29] Two G-rich sequences were assembled (**12** and **13**), observing in all cases coupling yields greater than 97%, on average, as evaluated by DMT test. The oligomers were detached from the support and deprotected by conc. aq. ammonia treatment as described for hybrids **8**–**11**. The supernatant was filtered and the support washed with $H_2O$. The combined filtrates and washings were concentrated under reduced pressure, redissolved in $H_2O$ and analyzed and purified by HPLC on a Nucleogel SAX column (Macherey-Nagel, 1000-8/46); buffer A: 20 mM $KH_2PO_4$ aq. solution, pH 7.0, containing 20% (v/v) $CH_3CN$; buffer B 1 M KCl, 20 mM $KH_2PO_4$ aq. solution, pH 7.0, containing 20% (v/v) $CH_3CN$; a linear gradient from 0 to 100% B in 20 min, flow rate 0.8 mL $min^{-1}$, for **12**, and in 40 min for **13** was used. The isolated oligomers, having the following retention times: **12** = 10.45 min; **13** = 16.62 min, were collected and successively desalted by gel filtration on a Sephadex G25 column eluted with $H_2O$/EtOH 4 : 1 (v/v). By HPLC analysis on a Nucleosil 100-5 $C_{18}$ analytical column (4.6 × 250 mm, 7 μm), the isolated oligomers were determined to be more than 98% pure. In the HLPC profile on the SAX column of both crudes, two main peaks were found, respectively at 10.45 and 15.30 min for **12**, and at 16.62 min and 27.16 min for **13**, which were all collected and desalted. As also reported by others for similar G-rich sequences,[33] in all the purification attempts by HPLC, the faster eluting peak, attributed to the single stranded 5-mer or 6-mer, was always accompanied by a major peak, having a higher retention time. This additional peak was attributed to a G-quadruplex structure generated under HPLC elution conditions. In fact, by re-injecting on HPLC the isolated and desalted faster eluting peak, the slower eluting peak was always observed; in addition, the two isolated peaks for each crude, analyzed by ESI-MS in several conditions, showed the same $m/z$ ion pattern. On the basis of this evidence, the peak at higher retention time has been attributed to a non-covalent adduct obtained by self-assembly of the monomeric oligomer. After lyophilization, the isolated compounds were characterized by ESI-MS data.

Compound **12**: ESI-MS: calculated mass: 1938; found 1938.28.

Compound **13**: ESI-MS: calculated mass: 2276; found 2276.50.

For comparison, $^{5'}$TGGGT$^{3'}$ and $^{5'}$TGGGAG$^{3'}$ were synthesized following standard solid phase protocols,[29] on a 5 μmol scale. Deprotection and HPLC purification of the oligomers were carried out as described above for **12** and **13**. The pure, isolated peaks were characterized by ESI MS, showing masses in accordance with the calculated values.

### Circular dichroism studies

The concentration of the conjugated ODNs was determined spectrophotometrically at $\lambda$ = 260 nm and at 90 °C, using the molar extinction coefficient calculated for the unstacked oligonucleotides using the following extinction coefficients: 15400 (A), 11700 (G) and 8800 (T) $cm^{-1}$ $M^{-1}$,[36] assuming the contribution of the sugar residues to the absorbance at $\lambda$ = 260 nm to be negligible. Compound **12** and 5-mer $^{5'}$TGGGT$^{3'}$ were dissolved at $c = 1.0 \times 10^{-4}$ M, while **13** and the parent 6-mer $^{5'}$TGGGAG$^{3'}$ were dissolved at $c = 3.3 \times 10^{-5}$ M in the buffer 10 mM $KH_2PO_4$, 1.00 M KCl and 0.1 mM EDTA. All the samples were first taken to 90 °C for 10 min, then slowly cooled to rt and kept at 5 °C overnight. CD spectra were then registered at 20 °C in a 0.1 cm pathlength cuvette. The wavelength was varied from 200 to 340 nm at 5 nm $min^{-1}$. CD spectra were recorded with a response of 16 s, at 2.0 nm bandwidth and normalised by subtraction of the background scan with buffer. The molar ellipticity was calculated from the equation $[\vartheta] = \vartheta/cl$ where $\vartheta$ is the relative intensity, $c$ the concentration of the oligonucleotide and $l$ is the pathlength of the cell in cm.

### Synthesis of conjugates 14 and 15 and of the parent oligonucleotides

ODN chain assembly was performed on 50 mg (3.5 μmol) of support **7** for **14** and on 50 mg of DMT-C-supported CPG (Millipore, High Loading, *ca.* 0.1 meq/g) for **15**. The syntheses were carried out on an automated DNA synthesizer following a standard phosphoramidite protocol with final DMT removal.[29] Two successive coupling cycles (30 min each) with phosphoramidite **5** were carried out as the last step of the assembly of **15**. The oligomers were detached from the support and deprotected by conc. aq. ammonia treatment as described for hybrids **8**–**13**. The supernatant was filtered and the support washed with $H_2O$. The combined filtrates and washings were concentrated under reduced pressure, redissolved in $H_2O$ and analyzed and purified by HPLC on a Nucleogel SAX column (Macherey-Nagel, 1000-8/46); buffer A: 20 mM $KH_2PO_4$ aq. solution, pH 7.0, containing 20% (v/v) $CH_3CN$; buffer B 1 M KCl, 20 mM $KH_2PO_4$ aq. solution, pH 7.0, containing 20% (v/v) $CH_3CN$; a linear gradient from 0 to 100% B in 30 min, flow rate 0.8 mL $min^{-1}$, was used. The isolated oligomers, having the following retention times: **14** = 21.14 min; **15** = 22.65 min, were collected and successively desalted by gel filtration on a Sephadex G25 column eluted with $H_2O$. By HPLC analysis on a Nucleosil 100-5 $C_{18}$ analytical column (4.6 × 250 mm, 7 μm), the isolated oligomers were determined to be more than 98% pure.

Compound **14** MALDI-TOF MS (negative mode): found: *m*/*z* 5827.95 $(M - H)^-$.

Compound **15** MALDI-TOF MS (negative mode): found: *m*/*z* 5828.42 $(M - H)^-$.

For comparison, d($^{5'}$GCGTGCCTCCTCACTGGC$^{3'}$) and d($^{5'}$TTGCCAGTGAGGAGGCACGCAT$^{3'}$) were synthesized on a 5 μmol scale, following standard solid phase protocols.[29] Deprotection and HPLC purification of the oligomers were carried out as described above for **14** and **15**. The pure, isolated peaks were characterized by MALDI-TOF mass spectrometry, showing masses in accordance with the calculated values.

### Thermal denaturation experiments

The concentration of the synthesized ODNs was determined spectrophotometrically at $\lambda$ = 260 nm and at 85 °C, using the molar extinction coefficient calculated for the unstacked oligonucleotide using the following extinction coefficients: 15400 (A); 11700 (G); 7300 (C); 8800 (T) $cm^{-1}$ $M^{-1}$.[36] For oligomers **14** and **15**, the contribution of the carbohydrate residues to the absorbance at $\lambda$ = 260 nm was considered negligible. A 100 mM NaCl, 10 mM $NaH_2PO_4$, aq solution at pH = 7.0 was used for the melting experiments. Melting curves were recorded using a concentration of approximately 1 μM for each strand in 1 mL of the tested solution in Teflon stoppered quartz cuvettes of 1 cm optical path length. The resulting solutions were then allowed to heat at 85 °C for 10 min, then slowly cooled and kept at 5 °C for 30 min. After thermal equilibration at 10 °C, UV absorption at $\lambda$ = 260 nm was monitored as a function of the temperature, increased at a rate of 0.5 °C/min, in the range 20–80 °C. The melting temperatures, determined as the maxima of the first derivative of absorbance *vs.* temperature plots, are the average values of three independent melting experiments. For all the studied cases, both the modified duplexes and the unmodified one, within the experimental error evaluated as ±0.5 °C, exhibited a melting temperature value of 60 °C.

## Acknowledgements

We thank MIUR (PRIN) for grants in support of this investigation and Centro di Metodologie Chimico-Fisiche (CIMCF), Università di Napoli "Federico II", for the NMR and MS facilities.

## References

1 E. Uhlmann and A. Peyman, *Chem. Rev.*, 1990, **90**, 543–584.
2 N. T. Thuong and C. Hélène, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 666–690.
3 M. Famulok, G. Mayer and M. Blind, *Acc. Chem. Res.*, 2000, **33**, 591–599.
4 Y. S. Sanghvi, in *Carbohydrate Modifications in Antisense Research*, ed. P. Dan Cook, ACS Symposium Series, Washington, USA, 1994.
5 S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1993, **49**, 1925–1963.
6 P. Virta, J. Katajisto, T. Niittymaki and H. Lonnberg, *Tetrahedron*, 2003, **59**, 5137–5174.
7 C. F. Brewer, M. C. Miceli and L. G. Baum, *Curr. Opin. Struct. Biol.*, 2002, **12**, 616–623.
8 M. W. Turner, *Mol. Immunol.*, 2003, **40**, 423–429.
9 H. Kunz, *Angew. Chem., Int. Ed. Engl.*, 1987, **26**, 294–308.
10 H. Paulsen, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 823–938.
11 B. G. Davis, *Chem. Rev.*, 2002, **102**, 579–601.
12 S. Akhtar, A. Roudledge, R. Patel and J. M. Gardiner, *Tetrahedron Lett.*, 1995, **36**, 7333–7336.
13 T. Ren and D. Liu, *Tetrahedron Lett.*, 1999, **40**, 7621–7625.
14 T. L. Sheppard, C.-H. Wong and G. F. Joyce, *Angew. Chem. Int. Ed.*, 2000, **39**, 3660–3663.
15 D. Forget, R. Renaudet, E. Defrancq and P. Dumy, *Tetrahedron Lett.*, 2001, **42**, 7829–7832.
16 M. A. Maier, C. G. Yannopoulos, N. Mohamed, A. Roland, H. Fritz, V. Mohan, G. Just and M. Manoharan, *Bioconjugate Chem.*, 2003, **14**, 18–29.
17 M. Dubber and J. M. J. Frechet, *Bioconjugate Chem.*, 2003, **14**, 239–246.
18 Y. Wang and T. L. Sheppard, *Bioconjugate Chem.*, 2003, **14**, 1314–1322.
19 K. Matsuura, M. Hibino, M. Kataoka, Y. Hayakawa and K. Kobayashi, *Tetrahedron Lett.*, 2000, **41**, 7529–7533.
20 K. Matsuura, M. Hibino, Y. Yamada and K. Kobayashi, *J. Am. Chem. Soc.*, 2001, **123**, 357–358.
21 S. Sando, K. Matsui, Y. Niinomi, N. Sato and Y. Aoyama, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 2633–2636.
22 K. Matsuura, M. Hibino, T. Ikeda, Y. Yamada and K. Kobayashi, *Chem. Eur. J.*, 2004, **10**, 352–359.
23 B. G. Davis, *J. Chem. Soc., Perkin Trans. 1*, 2000, 2137–2160.
24 P. H. Seeberger and W. Haase, *Chem. Rev.*, 2000, **100**, 4349–4394.
25 M. Adinolfi, G. Barone, L. De Napoli, L. Guariniello, A. Iadonisi and G. Piccialli, *Tetrahedron Lett.*, 1999, **40**, 2607–2610.
26 M. Adinolfi, L. De Napoli, G. Di Fabio, L. Guariniello, A. Iadonisi, A. Messere, D. Montesarchio and G. Piccialli, *Synlett*, 2001, **6**, 745–748.
27 M. Adinolfi, L. De Napoli, G. Di Fabio, A. Iadonisi, D. Montesarchio and G. Piccialli, *Tetrahedron*, 2002, **58**, 6697–6704.
28 N. Murakami, S. Tamura, E. Iwata, S. Aoki, S. Akiyama and M. Kobayashi, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 3267–3270.
29 *Oligonucleotides and Analogues: a Practical Approach*, ed. F. Eckstein, IRL Press, Oxford, 1991.
30 S. M. Kerwin, *Curr. Pharm. Des.*, 2000, **6**, 441–471.
31 M. A. Keniry, *Biopolymers*, 2001, **56**, 123–146.
32 R. Jin, B. L. Gaffney, C. Wang, R. A. Jones and K. J. Breslauer, *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 8832–8836.
33 H. Hotoda, M. Koizumi, R. Koga, M. Kaneko, K. Momota, T. Ohmine, H. Furukawa, T. Agatsuma, T. Nishigaki, J. Sone, S. Tsutsumi, T. Kosaka, K. Abe, S. Kimura and K. Shimada, *J. Med. Chem.*, 1998, **41**, 3655–3663.
34 This ODN sequence is complementary to a tract of mRNA of protein kinase A type 1 (PKA1), subunit RIα, overexpressed in the majority of human cancers and well recognized as a relevant target for therapeutic intervention against neoplasia; therefore several modifications of this sequence have been designed for antisense experiments and are currently under clinical trial. See for example: G. Tortora, R. Bianco, V. Damiano, G. Fontanini, S. De Placido, R. Bianco and F. Ciardiello, *Clin. Cancer Res.*, 2000, **6**, 2506–2512.
35 R. Khan, *Carbohydr. Res.*, 1974, **32**, 375–379.
36 *Handbook of Biochemistry and Molecular Biology*, Volume I, Nucleic Acids, ed. G. D. Fasman, 3rd edn., CRC Press, 1975, p. 589.